65 Modeling of the whole CFTR 3D structure and its conformational transitions

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The experimental 3D structure of the bacterial Sav1866 protein has provided invaluable insights into the structural basis underlying the function of ATP-Binding Cassette (ABC) exporters. Using this structure, we recently modeled the structure of the membrane-spanning domains (MSD1/MSD2) and nucleotide-binding domains (NBD1/NBD2) of human CFTR, in an ATP-bound, outward-facing conformation (Cell Mol Life Sci 2008 65:2594–612). This modeling, based on the use of sensitive methods allowing accurate alignments in the MSDs at high level of sequence divergence, highlighted the crucial roles of some amino acids within the MSDs (for substrate translocation) and at the interface between the MSDs and NBDs (coupling interfaces).

We next modeled the inward-facing conformation of human CFTR, based on the more recent corrected structures of the bacterial ABC lipid flippase MsbA.

This modeling provides a framework to understand the probable conformational transition between the inward- and outward-facing conformations, involving a large conformational rearrangement within the MSDs and an evolution of NBDs from a close conformation, in which the P-loops face each other, towards a tight interface, with the nucleotide being sandwiched between the P-loop and the signature sequence. Noteworthy is that the coupling interfaces between MSDs and NBDs are largely maintained, supporting their crucial role as constant pivots and highlighting the importance of F508 in this context.

In addition, we also constructed a two-segment model of the regulatory R domain. The so-deduced model of the whole MSDs:NBDs:R assembly is supported by the recent 3D reconstruction of CFTR from negatively stained electron microscopy images.

Supported by: Vaincre La Mucoviscidose.

66 Refinement of CFTR transmembrane segments by a novel prediction tool

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CFTR is an ATP-binding cassette transporter and respect the general topology of this super-family, which consists on two transmembrane domains (TMD) and two nucleotide-binding domains, plus a unique R domain. The two TMDs are both constituted of six transmembrane segments (TMS). They compose the CFTR pore, which allows ionic movements, assuming the main role of CFTR. The global topology of CFTR is well known, but these segments are roughly defined; their exact borders are still to be determined. Conduction and selectivity of CFTR pore is crucial for its main function and some severe mutations have already been found in TMD (p.Gly85Glu, p.Arg347Pro. . .). Better definition of the amino acids of TMS is obviously linked to an improvement in prediction of pathogenicity of sequence variations.

Crystallization of CFTR should provide crucial information on its structure and therefore on its mechanism. Due to CFTR high complexity and technical limitations, crystallization of its TMDs has not been achieved yet. To circumvent these restrictions, a great number of TMS prediction tools have been developed, but their predictions for CFTR do not exactly match; they generally agree on a hydrophobic core of a dozen of amino acids, which only represents about one half of a TMS.

We developed a bioinformatics tool for TMS and transmembrane secondary structure refinement based on a new process on hydrophobic data. We validated our tool on a set of well-defined channels such as glycerol-3-phosphate transporter. We will use this tool to improve knowledge on CFTR TMD and detect structural crucial amino acids. As soon as data from expert laboratories are collected, cured and integrated in the UMD-CFTRbase, results from our tool are expected to enhance UMD-CFTRbase predictions of sequence variations.

Supported by: VLM.

67* Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770

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Cystic fibrosis (CF) is a fatal genetic disease caused by mutations in the gene encoding CFTR, a PKA-gated Cl^- channel that regulates salt and fluid transport in epithelial tissues. In the lung, mutations that decrease CFTR-mediated Cl^- secretion result in increased Na^+ absorption through the epithelial Na^+ channel, ENaC. These abnormalities in salt transport are thought to dehydrate the airway surface, resulting in the deleterious cascade of mucus accumulation, infection, and inflammation that causes progressive lung damage. A therapeutic strategy for CF is to restore defective CFTR-mediated Cl^- secretion. A central question of this approach is whether pharmacological modulation of CFTR can restore the downstream defects in Na^+ and fluid transport sufficiently to promote mucociliary clearance. In these in vitro studies, we demonstrate that VX-770, a CFTR potentiator in clinical development to treat CF, increased Cl^- secretion in cultures of human bronchial epithelia (HBE) isolated from the airway of a subject with CF carrying the G551D and F508del CFTR mutations. The level of G551D/F508del-CFTR-mediated Cl^- secretion in the presence of VX-770 was sufficient to decrease the excessive Na^+ and fluid absorption and increase cilia beating. These data support the hypothesis that increasing CFTR-mediated Cl^- secretion with a small molecule may have pleiotropic effects on epithelial cell function in patients with CF who carry the G551D mutation.

Supported by: CFFT, Vertex Pharmaceuticals.

68* Rescue of A455E CFTR by temperature, small molecule correctors and transcomplementation

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A455E-CFTR is associated with milder forms of CF. Previous studies have shown that the single channel properties of A455E are normal but that reduced processing of the mutant channel is the likely cause of the mild disease phenotype (Sheppard et. al. EMBO Journal, 14:876–883, 1995). Given that A455E is within NBD1 and is a partial processing mutant we asked whether band C of A455E could be rescued similar to ΔF508-CFTR. To address this question we expressed A455E in Cos7 cells and observed that the steady state levels of band C were higher than observed for ΔF508-CFTR but lower than wt CFTR consistent with A455E being a partial processing mutant. Interestingly, A455E was strongly temperature sensitive, because growing cells at 27ºC has a large effect on increasing bands B and C. Steady state levels of band C of A455E were increased by treating cells with the proteasome inhibitors MG132 and PS341 suggesting that A455E is degraded in the proteasome. Treating cells with cycloheximide demonstrated that the half life of A455E is much longer than that of ΔF508-CFTR but less than wild type CFTR. Importantly, the corrector 4A (Pedemonte et. al. JCI, 115:2564–2571, 2005) also increased the steady state levels of bands B and C. We showed previously that a truncated form of CFTR, Δ264-CFTR, can rescue ΔF508-CFTR. Cotransfection with Δ264-CFTR also increased band C of A455E by transcomplementation. These results suggest that partial trafficking mutants of CFTR may also be likely candidates for small molecule corrector and gene therapies.

Supported by: CFF and NIH